Table I. Sesquiterpenes Characterized in the Steam Volatile Oil of Carrot Roots

	Rel % of hydrocarbon fraction	Rel % of whole oil
α-Bergamotene	0.4	0.2
Caryophyllene	8.8	5.1
β-Farnesene	0.5	0.3
α-Humulene	0.5	0.3
γ-Muurolene[a]	0.5	0.3
γ-Bisabolene (A)[b]	0.4	0.2
γ-Bisabolene (B)[c]	11.3	6.7

[a] Tentative. [b] γ-Bisabolene (A) tentatively assigned as *Z* isomer. [c] γ-Bisabolene (B) tentatively assigned as *E* isomer.

identified caryophyllene with 5.1% of the whole oil.

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Formation of Heterocyclic Compounds from the Reaction of L-Rhamnose with Ammonia

Takayuki Shibamoto and Richard A. Bernhard*

The reaction of L-rhamnose with ammonium hydroxide in an aqueous system was investigated, and 95 volatile reaction products were isolated using continuous liquid–liquid extraction with dichloromethane for 16 h. Qualitative and quantitative analyses of the reaction products were made using GC–MS techniques; 65 compounds were positively identified and 20 others tentatively identified. The principal constituents of the extracts were pyrazines, pyrroles, and imidazoles. Eight compounds hitherto unreported to be present in foods or sugar–amine model systems were identified. These are: 2-methyloxazole, 2-ethylpyrrole, 2-ethyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 4,5-dimethyloxazole-2-carboxaldehyde, 5-methyl-5*H*-cyclopentapyrazine, 2-ethyl-5*H*-cyclopentapyrazine, 2-ethyl-3-methyl-5,8-dihydroquinoxaline, and 2-amino-5-methylpyridine. A reaction mechanism for the formation of cyclic pyrazines featuring addition of ammonia to cyclopentenone derivatives and subsequent condensation with α-amino carbonyl compounds is advanced, as is also a possible mechanism for the formation of imidazoles from α-amino carbonyl compounds and carboxylic acids.

It is a well-known fact that the reaction of sugars with aqueous ammonia produces many heterocyclic compounds which include pyrazines, imidazoles, pyridines, and piperazines (Rizzi, 1974; van Praag et al., 1968; Jezo and Luzak, 1966). The reaction products of sugars and amine compounds have been studied intensively over the last several years, since pyrazines were recognized as important flavor constituents of a variety of roasted or toasted foods (Guadagni et al., 1972). Formation pathways for those heterocyclic compounds have been proposed by many researchers (Rizzi, 1974; Shibamoto and Bernhard, 1977 a,b; Velisek et al., 1976). The present view among most investigators suggests that there are two possible formation pathways. One is that the decomposition of sugars produces unstable α-diketones and aldehydes and that those carbonyls subsequently react with amines to form heterocyclic compounds (Walradt et al., 1971). The other is that sugars react with amines followed by formation of α-amino carbonyl intermediates, and these intermediates produce heterocyclic compounds (Jezo and Luzak, 1966). Recently, Shibamoto and Bernhard (1977b) reported the proposed formation pathways of 12 alkylpyrazines which were isolated from various sugar (D-glucose, L-rhamnose, 2-deoxy-D-glucose)–ammonia model systems and the authors' investigations support the second hypothesis. Ten α-amino carbonyl intermediates were proposed in order to explain the formation pathways of pyrazines from the sugar–ammonia model systems. The present paper covers

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Table I. Compounds Isolated from L-Rhamnose–Ammonia Model System

Peak no.[a]	Compound	I_{un}[b]	I_k[c]	Area, %	References
1.	Acetaldehyde	701	690	1.02	Mussinan and Walradt (1974)
2.	Acetone	812	819	0.91	Mussinan and Walradt (1974)
3.	Ethyl acetate	876	875	0.91	Mussinan and Walradt (1974)
4.	Dichloromethane (solvent)				
5.	2-Butanone	912	905	0.11	Mussinan and Walradt (1974)
6.	*n*-Propyl acetate	958	965	0.28	Index of Mass Spectral Data (1969)
7.	Azirine (tentative)	992		0.02	
8.	2-Methyloxazole	1157	1157	0.15	
9.	2,4,5-Trimethyloxazole	1226	1220	0.05	Mussinan and Walradt (1974)
10.	Pyrazine	1210	1214	0.32	Mussinan and Walradt (1974)
11.	2-Methylpyrazine	1252	1254	12.22	Mussinan and Walradt (1974)
12.	2,5-Dimethylpyrazine	1320	1321	20.18	Mussinan and Walradt (1974)
13.	2,6-Dimethylpyrazine	1324	1325	14.85	Mussinan and Walradt (1974)
14.	2-Ethylpyrazine	1328	1329	2.23	Mussinan and Walradt (1974)
15.	2,3-Dimethylpyrazine	1336	1336	2.05	Mussinan and Walradt (1974)
16.	2-Ethyl-6-methylpyrazine	1385	1388	3.95	Mussinan and Walradt (1974)
17.	2-Ethyl-5-methylpyrazine	1391	1396	3.75	Mussinan and Walradt (1974)
18.	Trimethylpyrazine	1397	1400	6.02	Mussinan and Walradt (1974)
19.	2-Ethyl-3-methylpyrazine	1400	1406	0.02	Bondarovich et al. (1967)
20.	2-Ethyl-3,5-dimethylpyrazine	1451	1449	0.16	Bondarovich et al. (1967)
21.	2,6-Diethylpyrazine	1454	1453	3.46	Bondarovich et al. (1967)
22.	2,5-Diethylpyrazine	1457	1457	0.02	Mussinan and Walradt (1974)
23.	2-Ethyl-3,5-dimethylpyrazine	1463	1460	6.40	Friedel et al. (1971)
24.	2-Methyl-n-propylpyrazine (tentative)	1465		0.11	
25.	Tetramethylpyrazine	1471	1471	1.00	Mussinan and Walradt (1974)
26.	2,3-Diethyl-5-methylpyrazine	1485	1494	0.08	Mussinan and Walradt (1974)
27.	2,5-Diethyl-3-methylpyrazine	1498	1501	0.19	Mussinan and Walradt (1974)
28.	2-Acetylfuran	1500	1500	0.44	Mussinan and Walradt (1974)
29.	Pyrrole	1509	1504	3.75	Budzikiewicz et al. (1964)
30.	2-Ethyl-3,5,6-trimethylpyrazine	1515	1510	0.50	Friedel et al. (1971)
31.	2,6-Diethyl-3,5-dimethylpyrazine (tentative)	1532		0.03	Walradt et al. (1971)
32.	2,5-Diethyl-3,6-dimethylpyrazine (tentative)	1546		0.53	Walradt et al. (1971)
33.	2-Methylpyrrole	1549	1554	0.78	Budzikiewicz et al. (1964)
34.	2,4-Dimethylpyrrole (tentative)	1553		0.09	Budzikiewicz et al. (1964)
35.	2,3-Dimethylpyrrole (tentative)	1574		0.06	Budzikiewicz et al. (1964)
36.	Pyrrole-1-carboxaldehyde	1590	1591	0.44	Walradt et al. (1971)
37.	1-Methylpyrrole-2-carboxaldehyde (tentative)	1603		0.09	Walradt et al. (1971)
38.	2-Ethylpyrrole	1612	1615	0.13	
39.	5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine	1633	1627	0.05	Mussinan and Walradt (1974)
40.	6,7-Dihydro-5*H*-cyclopentapyrazine	1647	1650	0.05	Mussinan and Walradt (1974)
41.	2,5-Dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1653	1657	0.25	Mussinan and Walradt (1974)
42.	3,5-Dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1669	1670	0.13	Vitzthum and Werkhoff (1975)
43.	2-Acetonylpyridine (tentative)	1689		0.05	
44.	5,8-Dimethyl-5,6,7,8-tetrahydroquinoxaline	1700	1704	0.27	Pittet et al. (1974)
45.	2-Ethyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	1702	1706	0.02	
46.	2-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine	1704	1706	0.01	Pittet et al. (1974)
47.	5-Ethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1710	1713	0.02	Pittet et al. (1974)
48.	5-Methyl-5,6,7,8-tetrahydroquinoxaline	1727	1729	0.10	Pittet et al. (1974)
49.	5,7-Dimethyl-5,6,7,8-tetrahydroquinoxaline	1738	1742	0.75	Pittet et al. (1974)
50.	Acetamide	1740	1740	0.02	Ferretti et al. (1970)
51.	5,6,7,8-Tetrahydroquinoxaline	1745	1745	-	Pittet et al. (1974)
52.	2,3,5-Trimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1757	1757	0.05	Pittet et al. (1974)
53.	2-Methyl-5,6,7,8-tetrahydroquinoxaline	1760	1761	0.02	Pittet et al. (1974)
54.	2-Ethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1762	1765	0.02	Pittet et al. (1974)
55.	2,3-Dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1770	1771	0.02	Pittet et al. (1974)
56.	2-Ethyl-5,6,7,8-tetrahydroquinoxaline	1777	1780	0.01	Vitzthum and Wekhoff (1975)
57.	2-Ethyl-5-methyl-5,6,7,8-tetrahydroquinoxaline (tentative)	1784		0.01	
58.	Propionamide	1793	1791	0.07	Ferretti et al. (1970)
59.	2-Ethyl-6-methyl-5,6,7,8-tetrahydroquinoxaline (tentative)	1801		0.05	
60.	2-Acetyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine (tentative)	1840		0.06	
61.	A pyrazine derivate (mol wt 138)	1856		0.05	
62.	Unknown	1861		0.07	
63.	2,3-Dimethyl-5,6,7,8-tetrahydroquinoxaline	1865	1863	0.02	Pittet et al. (1974)
64.	A pyrazine derivate (mol wt 152)	1887		0.03	
65.	A pyrazine derivate (mol wt 152)	1890		0.17	
66.	4,5-Dimethyloxazole-2-carboxaldehyde	1898	1896	0.20	
67.	Quinoxaline	1900	1901	0.08	Mussinan and Walradt (1974)
68.	6-Methylquinoxaline	1925	1930	0.07	Mussinan and Walradt (1974)
69.	Unknown	1941		0.07	
70.	2-Acetyl-4,5-dimethyloxazole (tentative)	1976		0.04	

Table I. *(Continued)*

71.	2-Hydroxymethyl-3,5-dimethylpyrazine (tentative)	1985		0.05	
72.	An oxazole derivate (mol wt 125)	1989		0.04	
73.	Unknown	1993		0.03	
74.	Pyrrole-2-carboxaldehyde	1998	2000	0.10	Walradt et al. (1971)
75.	2-Propylpyrrole	2001	2001	0.05	Walradt et al. (1971)
76.	2-Acetylpyrrole	2005	2001	0.18	Budzikiewicz et al. (1964)
77.	5-Methyl-5*H*-cyclopentapyrazine	2029	2026	0.19	
78.	2-Ethyl-3-methyl-5,6,7,8-tetrahydroquinoxaline (tentative)	2045	2047	0.07	
79.	2-Ethyl-5*H*-cyclopentapyrazine	2054	2054	0.16	
80.	Unknown	2064		0.20	
81.	2-Ethyl-5,8-dihydroquinoxaline	2085	2083	0.05	
82.	2-Acetonylpyrrole (tentative)	2092		0.05	
83.	2-Acetonyl-5-methylpyrrole (tentative)	2103		0.05	
84.	5-Propylpyrrole-2-carboxaldehyde (tentative)	2119		0.13	
85.	2-Ethyl-3-methyl-5,8-dihydroquinoxaline	2124	2119	0.10	
86.	Imidazole	2152	2149	1.59	Bowie et al. (1967)
87.	1-Ethyl-2-acetylimidazole (tentative)	2170		0.10	
88.	Unknown	2181		0.05	
89.	2-Methylimidazole	2192	2192	0.76	Bowie et al. (1967)
90.	1-Acetyl-4-methylimidazole (tentative)	2201		0.20	Fuchs and Sundell (1975)
91.	2,4-Dimethylimidazole	2209	2210	1.73	Jezo and Luzak (1963)
92.	An imidazole derivate (mol wt 138)	2215		1.27	
93.	2-Amino-5-methylpyridine	2234	2232	0.84	
94.	2-Ethylimidazole	2237	2237	0.60	Radziszewski (1883)
95.	Imidazole-2-carboxaldehyde	2259	2262	0.21	Bowie et al. (1967)
96.	2-Acetylimidazole (tentative)	2268		1.12	

[a] Peak no. in Figure 1. [b] Kovats indices of unknown compounds. [c] Kovats indices of known compounds.

Table II. Compounds of which Mass Spectra Have Not Been Published

Peak no.	Compound	MS fragmentation
38.	2-Ethylpyrrole	95 (40.1), 80 (100), 66 (23.7), 53 (27.6), 39 (32.9)
45.	2-Ethyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	162 (38.2), 161 (19.7), 147 (100), 133 (13.2), 39 (35.5)
77.	5-Methyl-5*H*-cyclopentapyrazine	132 (100), 131 (60.5), 52 (39.5), 39 (53.9)
79.	2-Ethyl-5*H*-cyclopentapyrazine	146 (76.3), 145 (100), 131 (3.3), 51 (19.7), 39 (19.7)
81.	2-Ethyl-5,8-dihydroquinoxaline	160 (67.1), 159 (100), 145 (6.6), 128 (14.5), 51 (7.9), 39 (14.5)
93.	2-Amino-5-methylpyridine	108 (100), 107 (27.6), 81 (14.5), 80 (63.2), 53 (26.3), 41 (22.4), 39 (17.1)

the isolation and identification of pyrazines (including those reported in previous papers), pyrroles, and imidazoles from an L-rhamnose–ammonia model system. The formation pathways of these heterocyclic compounds are also discussed.

EXPERIMENTAL SECTION

L-Rhamnose (Eastman) and ammonium hydroxide (Allied Chemicals) were obtained commercially. Authentic samples of pyrazines, pyrroles, and imidazoles were obtained from Ogawa & Co., Ltd., Tokyo, Japan.

Sample Preparation. A Kjeldahl flask (100 mL) containing an aqueous solution of 1 M L-rhamnose and 1 M ammonium hydroxide was cooled in an ice bath for 30 min. The neck of the flask was flame sealed and the ampule placed in an oven at 100 °C for 2 h. Volatile reaction products were isolated from the reaction mixture with 200 mL of dichloromethane using a continuous liquid–liquid extractor for 16 h. The dichloromethane extract was dried over anhydrous magnesium sulfate and solvent was removed using a rotary flash evaporator. Approximately 0.5 g of a brown viscous material was obtained. The qualitative and quantitative analyses of the reaction products were conducted following the gas chromatographic–mass spectrometric methods described by Shibamoto and Russell (1976). Unknowns were identified by comparison of their mass spectra and Kovats indices with those of authentic samples. Identities of compounds which were deduced from mass spectra, but for which no authentic samples are available, are indicated as tentatively identified. Compounds for which mass spectral data have not been published are listed in Table II with their mass spectral data.

RESULTS AND DISCUSSION

The compounds identified in the dichloromethane extract are listed in Table I in order of elution from the gas chromatographic column (Carbowax 20M), and a typical gas chromatogram is shown in Figure 1. The main constituents of this extract are pyrazines, pyrroles, and imidazoles. The percentages of their total gas chromatographic peak areas are 79.84, 5.38, and 4.89, respectively (excluding compounds only tentatively identified). The difference between the results obtained in the present study and those of the previous study (Shibamoto and Bernhard, 1977b) is due to the fact that in the present study a large number of cycloalkapyrazines (12 cyclopentapyrazines and nine quinoxalines), pyrroles, and imidazoles were recovered. This may be due to a difference in the extraction methods used in the two experiments. The continuous extraction method seems to recover a greater amount of the less volatile compounds (pyrroles, imidazoles), which were not recovered using a discontinuous extraction, i.e., using a separatory funnel (Tsuchida and Komoto, 1967). A comparison of the recovery of the main constituents between the previous and present studies is shown in Table III.

Other investigators have also found cycloalkapyrazines in various food products (Walradt et al., 1971; Mussinan and Walradt, 1974; Mussinan et al., 1973). Vitzthum and Werkhoff (1975) reported 17 alkylated five- and six-membered alicyclic pyrazines in roasted coffee. Walradt et al. (1974) proposed that the formation of these cyclic pyrazines is due to the reaction of 2-hydroxy-3-methyl-

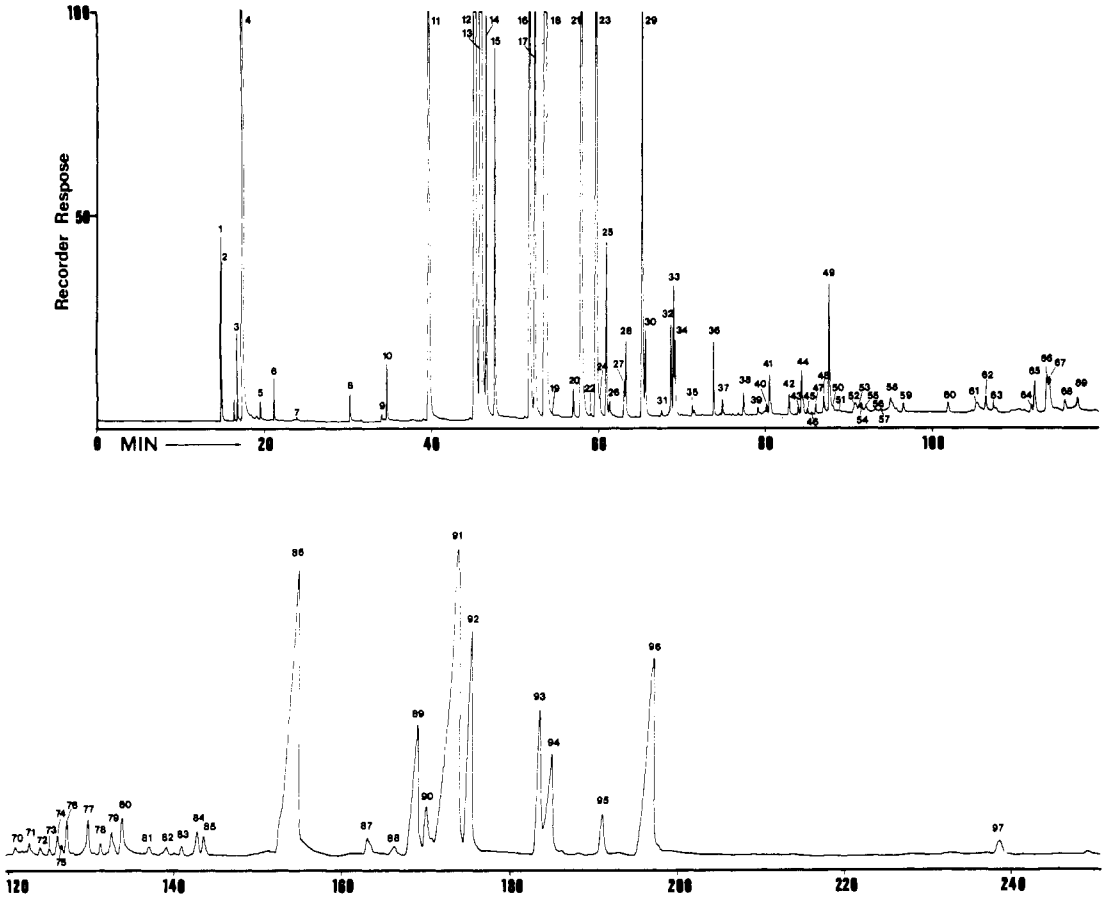


Figure 1. Gas chromatogram of products formed by the reaction of L-rhamnose (1 M) and ammonium hydroxide (1 M): column, 100 m × 0.25 mm i.d. glass capillary coated with Carbowax 20M, programmed from 70 to 170 °C at 1 °C/min and then held isothermally at 170 °C for 90 min; injector and detector temperature, 200 °C; chart speed, 12 in./h. Nitrogen carrier gas flow was 15 cm/s. See Table I for peak identification.

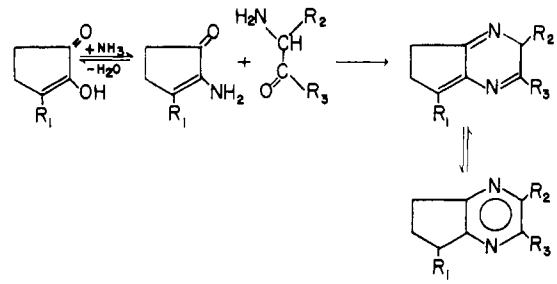
Table III. Percentages of Constituents in L-Rhamnose and Ammonia Reaction Mixture from Present and Previous Studies

Compound	Present, %	Previous, %[a]
Unsubstituted pyrazine	0.32	0.75
2-Methylpyrazine	12.22	20.02
2,5-Dimethylpyrazine	20.18	15.0
2,6-Dimethylpyrazine	14.85	22.4
2-Ethylpyrazine	2.23	5.16
2,3-Dimethylpyrazine	2.05	3.79
2-Ethyl-6-methylpyrazine	3.95	6.18
2-Ethyl-5-methylpyrazine	3.75	3.26
Trimethylpyrazine	6.02	11.83
2-Ethyl-3-methylpyrazine	0.02	5.83
2-Ethyl-3,6-dimethylpyrazine	0.16	5.85
2,6-Diethylpyrazine	3.46	*b*
2,5-Diethylpyrazine	0.02	*b*
2-Ethyl-3,5-dimethylpyrazine	6.40	4.35
Tetramethylpyrazine	1.00	*b*
Pyrrole	3.75	*b*
Imidazole	1.59	*b*
2,4-Dimethylimidazole	1.73	*b*

[a] Shibamoto and Bernhard (1977b). [b] Not detected.

2-cyclopenten-1-one, a product of carbohydrate degradation; dicarbonyl compounds; and amino acids. The details of the various formation mechanisms are, however, not yet clearly understood. Shibamoto and Bernhard (1977b) have previously proposed formation pathways for alkylpyrazines isolated from an L-rhamnose–ammonia model system. In their proposal, an α-amino carbonyl fragment was considered an important intermediate of pyrazine formation. The formation mechanism for cyclic

Scheme I



R_1: H, CH_3, C_2H_5
R_2: H, CH_3, C_2H_5
R_3: H, CH_3

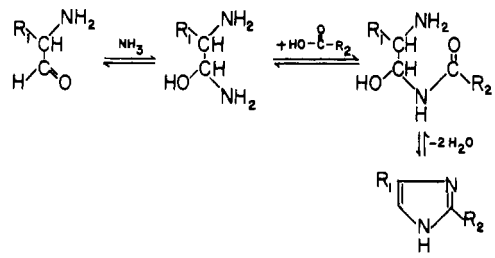
pyrazines as well as our previous formation scheme for alkyl pyrazines could be explained using the α-amino carbonyl intermediates shown in Scheme I.

Little information has been reported on the formation pathways for imidazoles. Jezo and Luzak (1966) discussed a possible formation pathway of imidazoles from the reaction of sucrose and ammonia. They suggested the presence of α-amino carbonyl fragments as intermediates of imidazole formation. Shibamoto and Bernhard (1977b) proposed a possible α-amino carbonyl fragment from the sugar–amine reaction which may be extended to hypothesize imidazole formation from a sugar–amine reaction as shown in Scheme II.

SUMMARY

As a result of investigation of the compounds isolated from the reaction of L-rhamnose and ammonia, 65 com-

Scheme II



R_1: H, CH_3
R_2: H, CH_3, C_2H_5, CHO

pounds have been positively identified and 20 compounds tentatively identified. Most compounds have been found in foods or sugar–amine model systems with the exception of 2-methyloxazole, 2-ethylpyrrole, 2-ethyl-5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 4,5-dimethyloxazole-2-carboxaldehyde, 5-methyl-5*H*-cyclopentapyrazine, 2-ethyl-5*H*-cyclopentapyrazine, 2-ethyl-3-methyl-5,8-dihydroquinoxaline, and 2-amino-5-methylpyridine.

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Flavor and Odor Thresholds in Water of Selected Orange Juice Components

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Odor and flavor thresholds in water were determined for four hydrocarbons, five alcohols, 13 aldehydes, six esters, and two ketones believed important to orange and other fruit flavors. In most cases no significant differences were found between odor and flavor threshold values. By correlating flavor threshold with level reported in orange juice, where available, the relative contribution of individual compounds to orange flavor was assessed. A comparison between these threshold values and previously reported threshold values in water showed generally good agreement with a few exceptions.

Although many common fruit owe their characteristic flavors to the aliphatic esters that are present, a major contribution to citrus flavors comes from the peel essential oil that contains mostly terpenoids. Over 90% of the essential oil of orange is the monoterpene hydrocarbon, *d*-limonene, but a major contribution to orange flavor is due to the minor oxygenated constituents, especially the aldehydes, esters, and alcohols (Kefford, 1959; Wolford and Attaway, 1967).

Well over 150 volatile compounds have been identified in orange juice or in flavor fractions derived from the juice (Shaw, 1977). Despite the extensive studies to identify volatile flavor components of orange juice, the primary flavor compound or mixture of compounds and their proportions needed for fresh orange flavor remain to be found. The quantities of most of the more abundant components present in orange juice have been estimated, but their significance to flavor has not been determined. Either odor or flavor thresholds have been determined for some volatile hydrocarbons, alcohols, aldehydes, and esters believed important to orange and to other fruit flavors (Berg et al., 1955; Buttery et al., 1971; Flath et al., 1967; Guadagni et al., 1963a; Lea and Swoboda, 1958). However, none of those studies reported a direct comparison between an odor threshold and a flavor threshold for a single compound. The values published for flavor and odor thresholds suffer from a lack of reproducibility, and no one study lists threshold values for most compounds believed important orange flavor. Since variations in threshold values are mainly due to differences in methodology (Pangborn, 1960), one study listing threshold values for all major volatile components of orange would be useful for determining relative flavor intensities, as well as providing flavor threshold values for components for which

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